

# Endocrine Measurements and Calving Performance of Swedish Red and White and Swedish Holstein Dairy Cattle with Special Respect to Stillbirth

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<sup>1</sup>Department of Obstetrics and Gynaecology, Centre for Reproductive Biology in Uppsala, <sup>2</sup>Department of Animal Breeding and Genetics, Swedish University of Agricultural Sciences, Uppsala, and <sup>3</sup>Swedish Dairy Association, Eskilstuna, Sweden.

**Kornmatitsuk B, Franzén G, Gustafsson H, Kindahl H: Endocrine measurements and calving performance of swedish red and white and swedish holstein dairy cattle with special respect to stillbirth. Acta vet. scand. 2003, 44, 21-33.** – During 3 consecutive calving seasons, calving performance, placental characteristics and endocrine profiles of total 98 pregnancies of late pregnant Swedish Red and White (SRB) and Swedish Holstein (SLB) dairy heifers and cows, were investigated. Ninety-four singleton pregnancies and 4 sets of twins were recorded. In animals with singleton pregnancy, 8 stillbirths, 7 weak calves, 3 premature parturitions and 1 abortion were registered. In the SLB heifers, 19% of stillbirth (5/26) were observed, while 5% (2/42) were noted for the SRB heifers. One stillborn calf derived from the SRB cows and none was found from the SLB cows. In the heifers and cows delivering a normal living calf with unassisted parturition, the placentome thickness monitored by ultrasonography was constant towards the end of pregnancy. The numbers of foetal cotyledons varied individually between animals but in total, fewer cotyledons were found in the foetal membranes of the SRB animals than in the SLB animals ( $69 \pm 19$ ) vs. ( $88 \pm 29$ ) ( $p < 0.05$ ). No morphological and numerical differences of the placentome thickness in animals delivering a stillborn or weak calf, compared to animals delivering a normal living calf, could be observed. In animals with unassisted parturition and without birth complications, the levels of progesterone (P4), PGF<sub>2α</sub> metabolite (PG-metabolite), cortisol, oestrone sulphate (E1SO4) and pregnancy associated glycoproteins (PAGs) were not different by breeds and parities. In animals carrying stillbirth, higher levels of E1SO4 were found in 3 SRB animals and 1 SLB heifer, whereas lower levels of E1SO4 were recorded in 3 SLB heifers during the last week of pregnancy, compared to the profiles found in animals with unassisted parturition. Additionally, the levels of PAGs remained low and constant in 1 SRB cow (delivering a stillborn calf), 1 SRB heifer (giving birth prematurely), 4 animals (carrying twins) and 1 aborting SRB cow. Our results show a very high rate of stillbirth in especially SLB heifers and deviating profiles of E1SO4 and PAGs in animals with impaired parturition were recorded.

***Cattle-pregnancy; parturition; endocrine profiles; calving performance; stillbirth.***

## Introduction

During late pregnancy and parturition in dairy cows, immense changes of endocrine parameters occur and exhibit tremendous influence on reproductive organs, involved in the normal

process of giving birth. Therefore, failures of foetal adaptation and improper synthesis and release of hormones at the end of pregnancy can possibly lead to calving difficulty, weak

calves and stillbirth, which contribute to the major losses of the calf at term.

In Swedish Holstein (SLB) heifers, close to 11% of the calves were stillborn, while in SLB cows and SRB heifers and cows about 5% were recorded (Swedish Dairy Association, 2001). Philipsson (1996) proposed that about half of stillbirth cases were from uncomplicated parturition with normal range of the calf body weight and Berglund (1996) found that slightly less than half of stillborn calves, sent for post mortem examinations, had signs of a difficult calving. Thereby, calf viability or foetal well-being might play a vital role in this situation. However, many other causes, which indicated conclusively this multifactorial problem, are involved for instance size of the dam, sex of the calf, gestation length, and sire used (Philipsson 1976, Thompson & Rege 1984, Berger et al. 1992, Meyer et al. 2000).

Mohamed et al. (1987) reported that the levels of progesterone (P4) could not predict the time of foetal death, whereas oestrone sulphate (E1SO4), oestrone in its conjugated form, might imply a status of the foeto-placental unit and placental viability (Dobson et al. 1993, Zhang et al. 1999). The analyses of pregnancy associated glycoproteins (PAGs), a group of placental glycoproteins, were previously successfully used for detection of early embryonic death in dairy cows (Zoli et al. 1992). They were suggested to be an initiating factor for the process of giving birth and an indicator of the status of foetal well-being (Patel et al. 1997, Beckers et al. 1999).

Thus, the aims of this study were to describe the calving performance of SRB and SLB dairy heifers and cows in a dairy herd with special respect to foetal viability and to reveal any associations of endocrine parameters to the evidence of stillbirth and related calving parameters.

## Materials and methods

### *Experimental design and animals used*

The study was done over 3 consecutive calving seasons at the research farm belonging to the Swedish University of Agricultural Sciences (SLU) in Uppsala. Totally 89 late pregnant dairy heifers and cows (6-7 months of pregnancy) were used. They were 51 Swedish Red and White (SRB) and 38 Swedish Holstein (SLB) breeds. In these numbers, 9 animals (7 SRB and 2 SLB) were examined in both parities 1 and 2 (parity 1 = heifers), thus in total 98 pregnancies were included. The animals were fed according to the Swedish standards (Spörndly 1993). The research farm was claimed free from bovine viral diarrhoea virus (BVDV) and bovine leukosis virus (BLV) infections. The plasma samples from animals with impaired parturition were tested for antibodies to *Neospora caninum* (Björkman et al. 1997). The care of the animals and the experimental design of this study were approved by the Local Animal Ethics Committee in Uppsala, Sweden.

### *Clinical observation and registration of the calving performance*

All animals were examined daily for health status and signs of approaching parturition by experienced barn people and the following parameters were recorded; date and time of parturition, degree of calving difficulty, calf sex and body weight, degree of calf viability and retained foetal membranes. An expected calving was estimated to take place 280 days after the last insemination. We considered an animal giving birth before 260 days of pregnancy with a living calf as an animal with premature parturition. Degree of calving difficulty was classified into 4 categories: 0 (unassisted), 1 (slight with light intervention), 2 (moderate with mild traction) and 3 (severe with heavy traction). Calving interventions were performed when

animals were suspected of having calving difficulty. Calf viability was scored in 3 degrees: healthy, weak and stillborn. The weak calf was determined by reduction of willingness to lift the head, purple appearance of mucous membranes and no attempt to escape from external stimuli. Stillbirth was defined as when, after at least 260 days of pregnancy, a calf died prior to, during or within 24 h after birth without evidence of infectious diseases. Abortion was considered when the animal delivered a dead foetus before 260 days of pregnancy. Foetal membranes were defined as retained (RFM) if not expelled within the first 24 h after delivery. The stillborn calves were autopsied at the Department of Pathology, the National Veterinary Institute in Uppsala, Sweden.

*Ultrasonography of the placentome thickness and gross examination of the foetal membranes*

All animals were subjected to ultrasound examinations, which were performed once weekly during the first calving season and every 3 weeks during the last 2 calving seasons. A real-time B-mode ultrasound (Aloca SSD-210 DXII, Tokyo, Japan) with a 5.0-MHz rectal linear array transducer, connected to a video-recording system was used. The basic principles of transrectal ultrasound technique described by *Pierson et al.* (1988) was applied. The measurement of the placentome thickness was done using an image freezer facility and electronic callipers of the equipment. In each examination, 1-5 placentomes were measured for the placentome thickness and the average value was calculated for further statistical analyses.

The expelled foetal membranes were kept at -20°C for investigation of gross appearance, evidence of infectious diseases, placental weight and the number of foetal cotyledons.

*Blood sampling and hormonal analyses*

Two blood sampling schemes were used in the study. The first scheme was performed during the first 2 calving seasons, once weekly blood sampling during the last 8 weeks prior to expected parturition until the parturition was completed. In the second scheme used in the last calving season, blood samples were taken more often; once weekly during 3-8 weeks before expected parturition and 3 times per week during the last 3 weeks prior to expected parturition until the parturition was completed. Blood samples were taken by jugular venipuncture into heparinized-evacuated tubes (Venoject, Terumo Europe N.V., Leuven, Belgium) and centrifuged immediately with  $2800 \times g$  for 20 min at 4°C. The plasma was separated into plastic tubes and stored at -20°C for hormonal analyses.

The hormonal assays were performed using radioimmunoassay (RIA) technique. The PGF<sub>2α</sub> metabolite (PG-metabolite) was determined according to *Granström & Kindahl* (1982). The procedures used for determinations of progesterone (P4), cortisol and oestrone sulphate (E1SO4) followed the manufacture's guidelines (Coat-A-Count Progesterone and Coat-A-Count Cortisol, Diagnostic Products Corporation, Los Angeles, CA, and DSL-5400, Diagnostic Systems Laboratories, Webster, TX, USA, respectively). The levels of pregnancy associated glycoproteins (PAGs) were assayed according to *Zoli et al.* (1992) at the Norwegian College of Veterinary Medicine, Oslo, Norway. The detection limits of each hormonal assay were given in the references mentioned above. The intra-assay and the inter-assay coefficients of variation of all hormonal analyses were below 10% and 14%, respectively.

*Statistical analyses*

The statistical analyses were performed by using the Statistical Analysis System (SAS), ver-

Table 1. The number of calving difficulty, stillbirth and weak calves in the singleton animals (sorted by breeds and parities).

Breed	Parity	N	Degree of calving difficulty (n)				Stillborn calves (n)	Weak calves (n)
			0	1	2	3		
SRB	1	42	37	3	2	0	2	3
	2	15	15	0	0	0	1	0
SLB	1	26	13	3	8	2	5	4
	2	11	10	1	0	0	0	0
Total		94	75	7	10	2	8	7

SRB = Swedish Red and White breed; SLB = Swedish Holstein breed; Parity 1 = heifers; N = the number of observations; Degree of calving difficulty: 0 = unassisted, 1 = slight, 2 = moderate, 3 = severe, n = number of cases.

sion 6.12 (SAS Institute Inc., Cary, NC, USA). The analyses of variance (ANOVA) were used for calculating significant differences among the means and the comparisons between 2 means were obtained using the modified t-test (Bonferroni method). The procedure for repeated measurements using PROC MIXED was employed for evaluating effects of variables (breed, time, parity and time  $\times$  parity) on the hormonal levels. Probability values of less than 0.05 were considered to be significant.

## Results

Out of the 98 pregnancies studied, 94 singleton

pregnancies (75 unassisted parturitions and 19 calving difficulties) and 4 sets of twins were registered. In animals with singleton pregnancies, 8 stillborn calves, 7 weak calves, 3 premature parturitions and 1 abortion were recorded. The premature parturitions derived from 3 SRB heifers, which gave birth on day 253, 255 and 258 of pregnancy, respectively and all calves were alive. The abortion occurring on day 233 of pregnancy came from a SRB cow. Descriptive data of the calving performance is presented in Tables 1-3.

The animals carrying twins gave birth on day 263, 272, 267 and 265 of pregnancy, respec-

Table 2. Descriptive data of the calving performance in animals delivering a stillborn calf.

No.	Breed	Parity	Gestation length (d)	Degree of calving difficulty				Calf sex / weight (kg)	Cotyl (n) / Placenta wt (kg)
				0	1	2	3		
1064	SRB	1	281			Yes		M / 43	73 / 4.6
1085	SRB	1	276		Yes			M / 39	45 / 2.8
1075	SRB	2	261	Yes				F / 39	RFM
1041	SLB	1	281			Yes		M / 54	83 / 3.9
1086	SLB	1	279				Yes	F / 44	56 / 2.0
1087	SLB	1	280			Yes		M / 54	99 / 4.5
1090	SLB	1	285	Yes				M / 50	70 / 3.8
1102	SLB	1	286			Yes		M / 47	RFM

SRB = Swedish Red and White breed; SLB = Swedish Holstein breed; Parity 1 = heifers; Degree of calving difficulty: 0 = unassisted, 1 = slight, 2 = moderate, 3 = severe; M = male, F = female; Cotyl. (n) = the number of cotyledons; Placenta wt = placental weight; RFM = retained foetal membranes.

Table 3. Descriptive data of the calving performance in animals delivering a weak calf.

No.	Breed	Parity	Gestation length (d)	Degree of calving difficulty				Calf sex / weight (kg)	Cotyl (n) / Placenta wt (kg)
				0	1	2	3		
1044	SRB	1	280			Yes		M / 47	30 / 2.5
1055	SRB	1	276		Yes			M / 45	65 / 4.8
1078	SRB	1	279		Yes			F / ?	?
1093	SLB	1	279		Yes			F / 47	91 / 3.5
1099	SLB	1	287		Yes			M / 43	96 / 4.1
1101	SLB	1	281				Yes	M / 56	37 / 3.2
1110	SLB	1	276			Yes		M / 52	95 / 5.6

SRB = Swedish Red and White breed; SLB = Swedish Holstein breed; Parity 1 = heifers; Degree of calving difficulty: 0 = unassisted, 1 = slight, 2 = moderate, 3 = severe; M = male, F = female; Cotyl. = the number of cotyledons; Placenta wt = placental weight; ? = a missing value (only the calf from No. 1078 died 24 - 48 h after birth)

tively. In total, 8 retained foetal membranes (RFM) were observed. They were from 2 animals with stillbirth, 2 animals carrying twins, 1 SRB heifer with premature parturition, 1 aborting SRB cow and 2 animals with unassisted parturition. The autopsy of the stillborn calves revealed no evidence of gross malformation and infectious diseases. Calving difficulty was indicated as a possible cause in 1 case (from a SLB heifer; No. 1087) due to severe trauma. One SRB cow (No. 1075) delivering a stillborn calf on day 261, was found positive for *Neospora caninum* antibodies (absorbance 0.41). The remaining animals were found negative.

#### *Ultrasonography of the placentome thickness and gross examination of the foetal membranes*

In animals with unassisted parturition and without other birth complications, the placentome thickness among breeds and parities was relatively constant during the study period. In addition, the size and shape of the placentome varied individually between animals. The average values of the placentome thickness in SRB heifers (n = 30) and cows (n = 10) were 2.9 cm  $\pm$  0.6 cm and 3.0 cm  $\pm$  0.6 cm, respectively and in SLB heifers (n = 9) and cows (n = 9) were 2.9

cm  $\pm$  0.6 cm and 3.1 cm  $\pm$  0.4 cm, respectively. No statistical differences of the placentome thickness were found between breeds and parities ( $p > 0.05$ ).

In animals delivering a stillborn or weak calf, the placentome thickness was also constant throughout the pregnancy. The average values of the placentome thickness in animals delivering a stillborn calf (n = 7) and a weak calf (n = 5) were 3.0 cm  $\pm$  0.4 cm and 3.3 cm  $\pm$  0.6 cm, respectively. There were no statistical differences of the placentome thickness between animals with unassisted parturition and animals delivering a stillborn or weak calf ( $p > 0.05$ ). Ultrasonography investigations without finding any placentomes were recorded in certain SRB and SLB animals both in animals with unassisted parturition and in animals delivering a stillborn or weak calf.

Due to practical reasons, only 58 complete foetal membranes were collected and examined. In this number, 40 complete foetal membranes were expelled from the singleton cows with unassisted parturition and without birth complications. The mean number of cotyledons and the placental weight in this group were 76 kg  $\pm$  24 kg and 3.3 kg  $\pm$  0.8 kg, respectively (details in different breeds and parities are shown

Table 4. The number of cotyledons and placental weight in the singleton animals with unassisted parturition and without birth complications. Data is sorted by breeds and parities.

Breed	Parity	No. of observation	No. of cotyledons		Placental weight (kg) (Mean $\pm$ SD)
			Mean $\pm$ SD	Min - Max	
SRB	1	20	70 $\pm$ 18	40 - 114	3.3 $\pm$ 0.7
	2	7	67 $\pm$ 23	41 - 103	3.0 $\pm$ 0.8
SLB	1	8	87 $\pm$ 31	47 - 134	3.7 $\pm$ 1.0
	2	5	90 $\pm$ 27	60 - 131	3.1 $\pm$ 1.0
Total		40	76 $\pm$ 24	40 - 134	3.3 $\pm$ 0.8

SRB = Swedish Red and White breed; SLB = Swedish Holstein breed; Parity 1 = heifers.

in Table 4). No gross abnormality or signs of infections were observed in any of the foetal membranes investigated.

The mean number of cotyledons and the placental weight in animals delivering a stillborn calf were 71 kg  $\pm$  19 kg and 3.6 kg  $\pm$  1.0 kg, respectively and the mean number of cotyledons and the placental weight in animals delivering a weak calf were 69 kg  $\pm$  30 kg and 3.9 kg  $\pm$  1.1 kg, respectively. Descriptive data of the placental characteristics in animals delivering a stillborn or weak calf, is presented individually in Tables 2-3. No significant differences of the number of cotyledons and the placental weight, compared between breeds, parities and groups were found ( $p > 0.05$ ). However, SRB animals had significantly fewer cotyledons (69  $\pm$  19) than SLB animals (88  $\pm$  29) ( $p < 0.05$ ) (Table 4).

#### *Endocrine changes during late pregnancy in SRB and SLB dairy heifers and cows*

##### *– With unassisted parturition and without birth complications*

During late pregnancy, the P4 levels gradually decreased toward the end in all animals and a pronounced decrease of P4 started 1 week prior to parturition. Collaterally, the levels of the PG-metabolite remained constant and the levels slightly increased during the last week of pregnancy. The levels of plasma cortisol fluctuated along the whole period of the study but an in-

crease of plasma cortisol was recorded towards the end of pregnancy. The levels of E1SO4 increased gradually and a significant rise was found on the day of parturition, whereas the levels of PAGs were very low and constant until the last 2 weeks prior to parturition and markedly increased when the animals were approaching parturition. The hormonal profiles during late pregnancy in SRB and SLB animals, sorted by parity are presented in Fig. 1.

From a statistical point of view, there was no difference of the hormonal levels between breeds and parities. The time factor significantly influenced the levels of all hormones ( $p < 0.001$ ). The time  $\times$  parity factor significantly affected the levels of P4, PG-metabolite, cortisol ( $p < 0.001$ ) and PAGs ( $p < 0.02$ ) but there was no effect on the levels of E1SO4.

##### *– With birth complications and respect to stillborn and weak calves*

No differences of P4, PG-metabolite and cortisol levels were observed between animals delivering a stillborn or weak calf and animals having unassisted parturition ( $p > 0.05$ ). Three SRB animals delivering a stillborn calf showed higher levels of E1SO4, compared to the profile found in SRB animals with unassisted parturition (Fig. 2; left upper panel). An increase of PAGs levels at the end of pregnancy was recorded in 2 SRB heifers but 1 SRB cow (No.

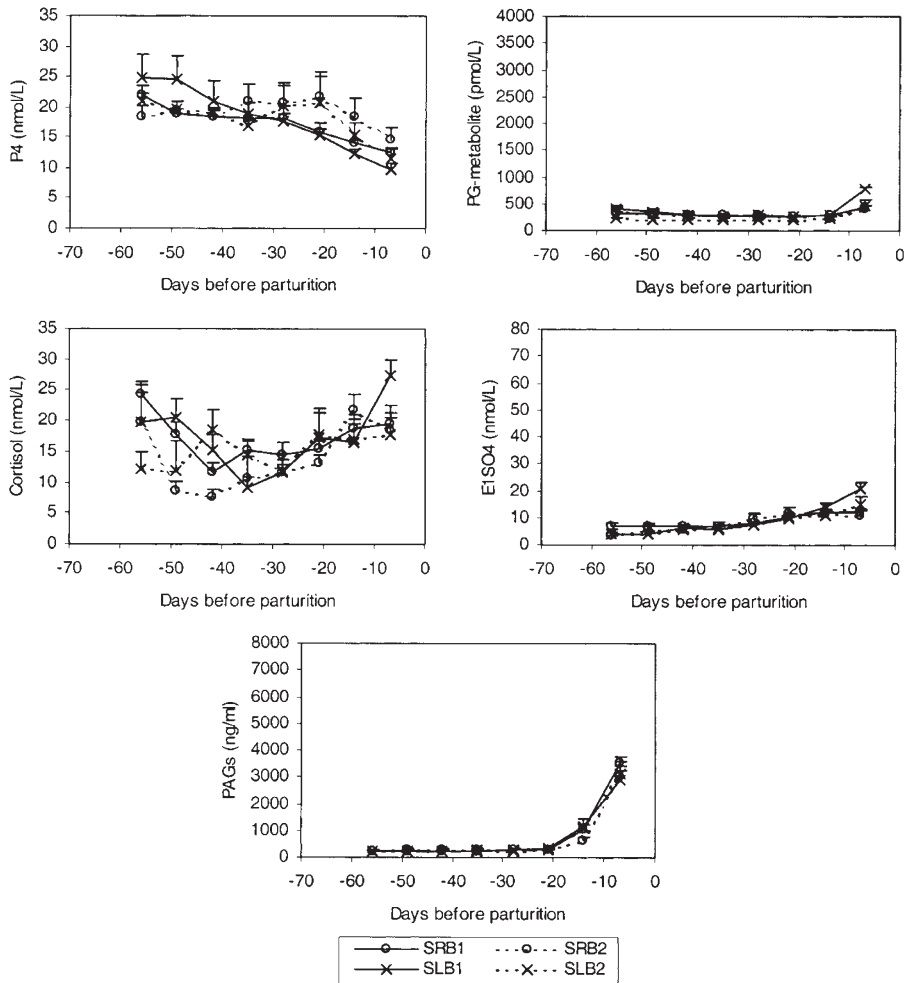


Figure 1. Hormone profiles during late pregnancy in SRB and SLB dairy heifers (SRB1 & SLB1) and cows (SRB2 & SLB2) with unassisted parturition and without birth complications (Means  $\pm$  SEM).

1075), which was found positive for *Neospora caninum* antibodies, showed constant PAGs levels (Fig. 2; right upper panel).

In 5 SLB heifers delivering a stillborn calf, 1 of them (No. 1041) showed a normal profile of E1SO4, compared to the profile in SLB heifers with unassisted parturition. Three heifers (No. 1086, 1090 and 1102) showed low levels of

E1SO4 during the last week of pregnancy and 1 heifer (No. 1087) showed a very irregular E1SO4 profile with high levels 50-60 d before parturition (Fig. 2; left lower panel). The levels of PAGs in these 5 SLB heifers followed the profile found in SLB heifers with unassisted parturition (Fig. 2; right lower panel).

All SRB and SLB heifers delivering a weak calf

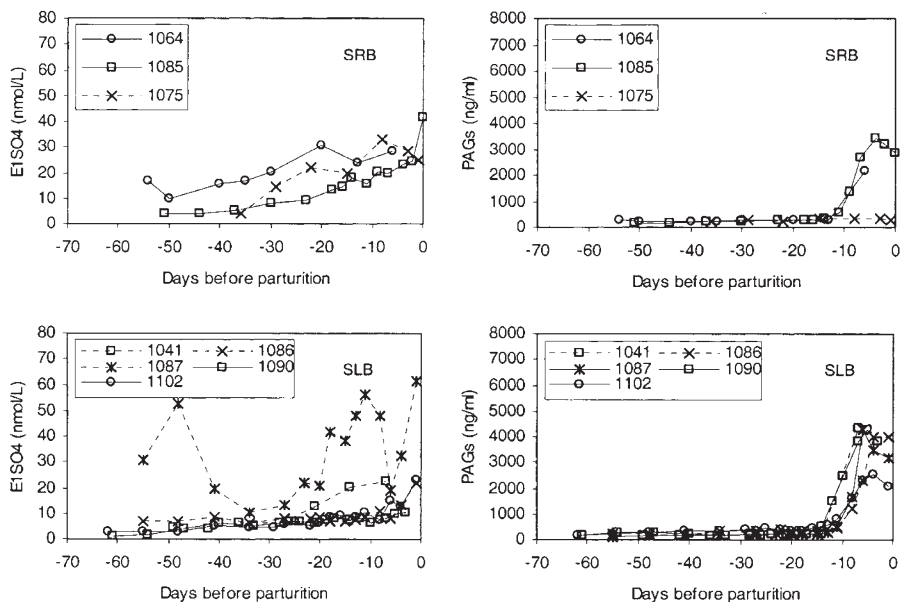


Figure 2. Plasma EISO4 and PAGs profiles in SRB and SLB animals delivering a stillborn calf.

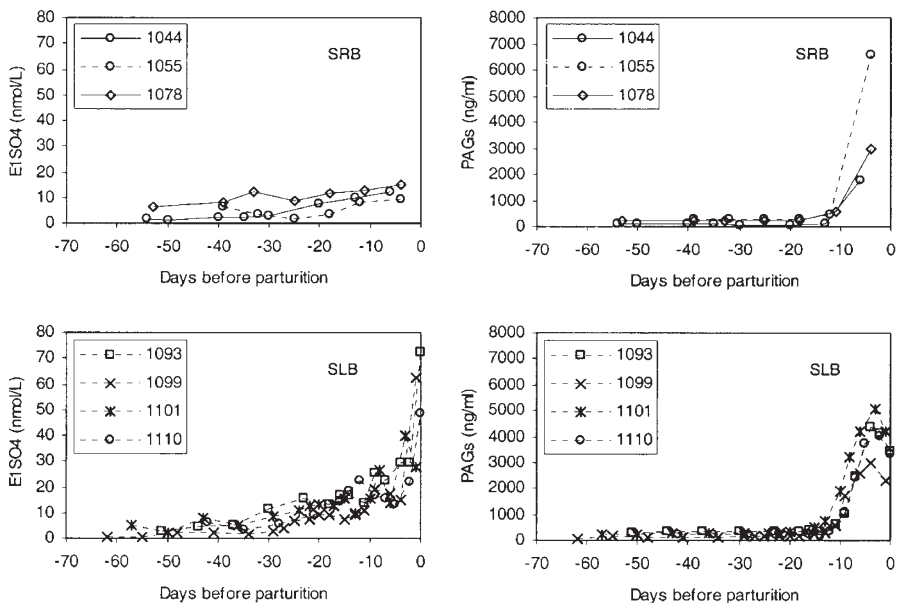


Figure 3. Plasma EISO4 and PAGs profiles in SRB and SLB animals delivering a weak calf.



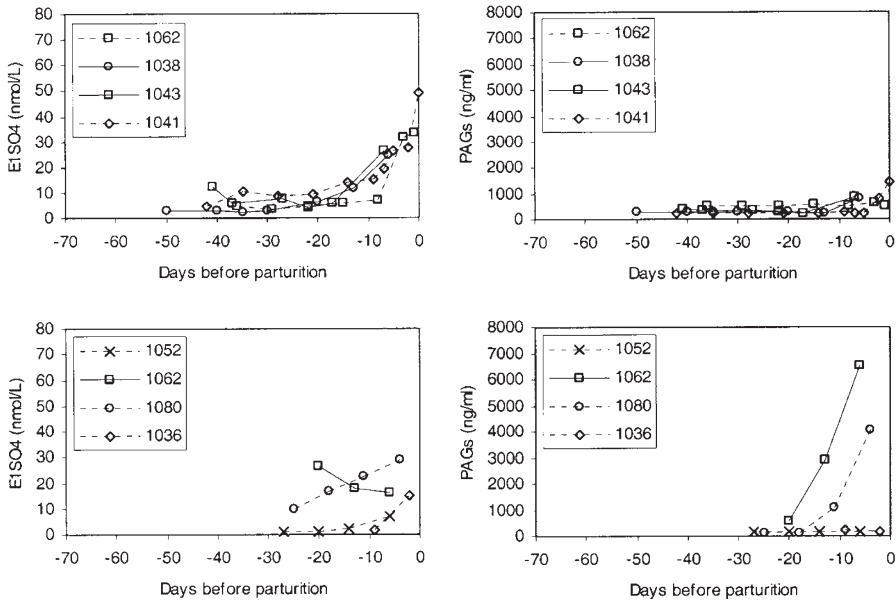


Figure 4. Plasma E1SO4 and PAGs profiles in 4 animals carrying twins (upper panels), 3 SRB heifers having premature parturition (lower panels; No. 1052, 1062 and 1080) and 1 aborting SRB cow (lower panels; No. 1036).

showed normal profiles of E1SO4, compared to the profiles found in SRB and SLB heifers with unassisted parturition but the levels of E1SO4 prior to parturition in SLB heifers increased with a higher magnitude (Figure 3; left panels). Increasing levels of PAGs in all SRB and SLB heifers delivering a weak calf prior to parturition were recorded and followed the profiles found in SRB and SLB heifers with unassisted parturition (Fig. 3; right panels). From a statistical point of view, the levels of E1SO4 in animals delivering a weak calf were significantly higher than the levels of E1SO4 in animals with unassisted parturition ( $p < 0.01$ ).

In 4 animals carrying twins, the increasing levels of E1SO4 were recorded during the last week of gestation, however, the levels of PAGs still remained low (Fig. 4; upper panels). In 3 animals with premature parturition and 1 abort-

ing SRB cow (No. 1036), an increase of E1SO4 prior to parturition was observed (Fig. 4; left lower panel). Low levels of PAGs, compared to the levels in animals with unassisted parturition, were recorded in 1 SRB heifer (No. 1052) with premature parturition and 1 aborting SRB cow as shown in Fig. 4; right lower panel.

## Discussion

The number of stillborn calves found in our group of SLB heifers (5/26, 19%) was higher than the 11% of stillbirth reported by the farmers in the milk recording system (Swedish Dairy Associations, 2001). This confirms that stillbirth is a serious problem for SLB heifers. Chassagne *et al.* (1999) proposed that several risk factors were related to the stillbirth for instance dystocia and a body condition score. Furthermore, Meyer *et al.* (2000 & 2001) indi-

cated that primiparous and multiparous cows clearly differed in the rate of stillbirth (almost twice in primiparous cows) and male calves tend to have a higher stillbirth rate than female calves (McDermott *et al.* 1992), which were also observed in our study. The increase of stillbirth rate in the SLB population is suggested to be associated with a high proportion of Holstein-Friesian genes due to importation of frozen semen from the North American Holstein Friesian bulls (Berghlund & Philipsson 1992, Steinbock *et al.* 2000 & 2002).

*Neospora caninum* is a well-known organism causing abortion in cattle and feeble calves at birth (Dubey & Lindsay 1993). In our results however, only one SRB cow was found positive and thus the parasite might not be a main reason for the high stillbirth rate found in this study.

The placentome thickness monitored by ultrasonography was in the same range (2-3 cm) as reported by Schlafer *et al.* (2000). The placental weight and the number of cotyledons found in our study were less than reported in the literature (varied between 4-5 kg and 70-120 cotyledons) (Peter & Ball 1995, Schlafer *et al.* 2000, Noakes *et al.* 2001). One explanation is that the foetal membranes, examined in our study, were frozen and some fluid was likely to have been removed after thawing, which reduced the placental weight. In addition, the placentome shrinkage may have caused some missing observations of small-sized placentomes. Nevertheless, we found a significant difference in the number of cotyledons between the 2 breeds. It is difficult to speculate about the significance of this finding in relation to calf viability since the breed with the lowest number of cotyledons (SRB) had the highest calf viability. The difficulty of finding the placentome by ultrasonography was obvious in a number of animals with both unassisted and impaired parturition. The position of the uterus and the location of the placentomes at the time of scan-

ning might of course influence the chance of finding placentomes. Another factor might be the vascularisation at the placentomes, which might cause lower echogenicity. It was too few observations in the impaired group to draw conclusions if the frequency was different, compared to the unassisted parturition.

The hormonal patterns during late pregnancy in dairy cows show typical profiles and they were suggested to be useful for monitoring of foetal well-being (Kindahl *et al.* 2002). Nevertheless, the determination of foetal death by use of P4 is not successful in all cases. Some investigators found the levels of P4 remained for several days or weeks after the foetal death or abortion. In our study, the evidence of stillbirth was not related to a drop of P4 or higher levels of PG-metabolite and cortisol. A change of E1SO4 has been proposed as a sensitive response variable indicative of conceptus function. However, variation among cows in circulating E1SO4 levels during late pregnancy may be caused by variation of placental development, oestrogen production and conjugation (Zhang *et al.* 1999) and the specific responses of maternal and foetal units (Thatcher *et al.* 1980). Lower levels of E1SO4 were possible causes of calving difficulty (Zhang *et al.* 1999) and were suggested to be associated with the occurrence of retained foetal membranes (Abdo *et al.* 1991). In our results, the levels of E1SO4 showed higher levels in SRB animals carrying stillbirth and lower levels in SLB animals delivering stillborn calves. These results indicate that E1SO4 plays an important role during late pregnancy and the analyses of E1SO4 might be a part of monitoring of foetal well-being particularly for SLB heifers.

The levels of PAGs progressively increased during late pregnancy and reached the peak around parturition (Zoli *et al.* 1992, Patel *et al.* 1997, Dosogne *et al.* 1999), which were also seen in our results with animals having unassisted par-

turition. This group of hormones was suggested to be involved in the initiation of parturition (Beckers *et al.* 1999). The peripheral increases of PAGs prior to delivery appeared at the same time when the marked degranulation of binucleate cells occurred (Schlafer *et al.* 2000). In our study, the peripartal levels of PAGs did not rise or were relatively low in the animals carrying twins or aborting. This finding suggests that the degranulation of PAGs is related to the gestation length (Kornmatitsuk *et al.* 2002) and might be also associated with the placental maturity. However, the entire process of peripartal degradation of PAGs is not thoroughly clear.

### Conclusions

This study showed a very high incidence of stillbirth in SLB heifers. No differences in the morphology of the foetal membranes were observed between animals with viable calves, compared to animals with stillbirth. In total, SRB animals had fewer cotyledons in the foetal membranes than SLB animals. Deviating profiles of EISO4 and PAGs in animals with impaired parturition were recorded. Additionally, low levels of EISO4 in 3 SLB heifers carrying stillbirth were observed.

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### References

- Abdo GA, Njuguna OM, Fredriksson G, Madej A: Levels of oestrone sulphate during pregnancy in different breeds of cows and its possible association with retained foetal membranes. *Acta Vet. Scand.* 1991, 32, 183-188.
- Beckers JF, Drion PV, Garbayo JM, Perenyi Z, Zarrouk A, Sulon J, Remy B, Szenci O: Pregnancy associated glycoproteins in ruminants: inactive members of the aspartic proteinase family. *Acta Vet. Hung.* 1999, 47, 461-469.
- Berger PJ, Cubas AC, Koehler KJ, Healey MH: Factors affecting dystocia and early calf mortality in Angus cows and heifers. *J. Anim. Sci.* 1992, 70, 1775-1786.
- Berglund B: Ongoing research on the causes of variation in calving performance and stillbirths in Swedish dairy cattle. *Interbull Bulletin* 1996, 12, 78-83.
- Berglund B, Philipsson J: Increasing stillbirth rate in the Swedish Holstein population. Paper in the 43rd EAPP Meeting, Madrid, Spain, 14-17 September 1992.
- Björkman C, Holmdahl OJM, Uggla A: An indirect enzyme-linked immunoassay (ELISA) for demonstration of antibodies to *Neospora caninum* in serum and milk of cattle. *Vet. Parasitol.* 1997, 68, 251-260.
- Chassagne M, Barnouin J, Chacornac JP: Risk factors for stillbirth in Holstein heifers under field conditions in France: a prospective survey. *Theriogenology* 1999, 51, 1477-1488.
- Dobson H, Rowan TG, Kippax IS, Humblot P: Assessment of fetal number, and fetal and placental viability throughout pregnancy in cattle. *Theriogenology* 1993, 40, 411-425.
- Dosogne H, Burvenich C, Freeman AE, Kehrli ME Jr, Detilleux JC, Sulon J, Beckers JF, Hoeben D: Pregnancy-associated glycoprotein and decreased polymorphonuclear leukocyte function in early post-partum dairy cows. *Vet. Immunol. Immunopathol.* 1999, 67, 47-54.
- Dubey JP, Lindsay DS: Neosporosis. *Parasitol. Today* 1993, 9, 452-458.
- Granström E, Kindahl H: Radioimmunoassay of the major plasma metabolite of PGF<sub>2α</sub>, 15-keto-13,14-dihydro-PGF<sub>2α</sub>. *Methods Enzymol.* 1982, 86, 320-339.
- Kindahl H, Kornmatitsuk B, Königsson K, Gustafsson H: Endocrine changes in late bovine pregnancy with special emphasis on fetal well-being. *Dom. Anim. Endocrinol.* 2002, 23, 321-328.

- Kornmatitsuk B, Veronesi MC, Madej A, Dahl E, Ropstad E, Beckers JF, Forsberg M, Gustafsson H, Kindahl H: Hormonal measurements in late pregnancy and parturition in dairy cows-possible tools to monitor foetal well being. *Anim. Reprod. Sci.* 2002, 72, 153-164.
- McDermott JJ, Allen OB, Martin SW, Alves DM: Patterns of stillbirth and dystocia in Ontario cow-calf herds. *Can. J. Vet. Rec.* 1992, 56, 47-55.
- Meyer CL, Berger PJ, Koehler KJ: Interactions among factors affecting stillbirths in Holstein cattle in the United States. *J. Dairy Sci.* 2000, 83, 2657-2663.
- Meyer CL, Berger PJ, Koehler KJ, Thompson JR, Sattler CG: Phenotypic trends in incidence of stillbirth for Holsteins in the United States. *J. Dairy Sci.* 2001, 84, 515-523.
- Mohamed AR, Noakes DE, Booth JM, Chaplin V: Plasma oestrone sulphate and progesterone concentrations in cows and ewes associated with fetal death and abortion. *Br. Vet. J.* 1987, 143, 238-245.
- Noakes DE, Parkinson TJ, England GCW: *Arthur's Veterinary Reproduction and Obstetrics* (eighth edition). W.B. Saunders Company Ltd. 2001.
- Patel OV, Sulon J, Beckers JF, Takahashi T, Hirako M, Sasaki N, Domeki I: Plasma bovine pregnancy-associated glycoprotein concentrations throughout gestation in relationship to fetal number in the cow. *Eur. J. Endocrinol.* 1997, 137, 423-428.
- Peter AR, Ball PJH: *Reproduction in cattle* (second edition). Blackwell Science Ltd. 1995.
- Philipsson J: Studies on calving difficulty, stillbirth and associated factors in Swedish cattle breeds. *Acta Agri. Scand.* 1976, 26, 151-164.
- Philipsson J: Strategies to reduce problems in calving performance and stillbirths by selection and differential use of bulls. *Interbull Bulletin* 1996, 12, 65-71.
- Pierson RA, Kastelic JP, Ginther OJ: Basic principles and techniques for transrectal ultrasonography in cattle and horses. *Theriogenology* 1988, 29, 3-20.
- Schlafer DH, Fisher PJ, Davies CJ: The bovine placenta before and after birth: placental development and function in health and disease. *Anim. Reprod. Sci.* 2000, 60-61, 145-160.
- Spörndly R: Fodertabeller för idisslare (Nutrition requirement for ruminants), Speciella skrifter 1993, 52. (in Swedish)
- Steinbock L, Johansson K, Näsholm A, Berglund B, Philipsson J: Direct and maternal genetic effects on stillbirths and dystocia in Swedish Holstein. Paper in the 51st EAAP Meeting, The Hague, The Netherlands, 21-24 August 2000.
- Steinbock L, Näsholm A, Berglund B, Johansson K, Philipsson J: Genetic effects on stillbirth and calving difficulty in Swedish Holsteins at first and second calving. 2002 (submitted).
- Swedish Dairy Association: *Husdjursstatistik* [Cattle statistics]. Svensk Mjölk, Eskilstuna, Sweden. 2001. (in Swedish)
- Thatcher WW, Wilcox CJ, Collier RJ, Eley DS, Head HH: Bovine conceptus-maternal interactions during the pre- and postpartum periods. *J. Dairy Sci.* 1980, 63, 1530-1540.
- Thompson JR, Rege JE: Influences of dam on calving difficulty and early calf mortality. *J. Dairy Sci.* 1984, 67, 847-853.
- Zhang WC, Nakao T, Moriyoshi M, Nakada K, Ribadu AY, Ohtaki T, Tanaka Y: Relationship of maternal plasma progesterone and estrone sulfate to dystocia in Holstein-Friesian heifers and cows. *J. Vet. Med. Sci.* 1999, 61, 909-913.
- Zoli AP, Guilbault LA, Delahaut P, Ortiz WB, Beckers JF: Radioimmunoassay of a bovine pregnancy-associated glycoprotein in serum: its application for pregnancy diagnosis. *Biol. Reprod.* 1992, 46, 83-92.

### Sammanfattning

*Hormonella mätningar och kalvningsförlopp hos mjölkdjur av Svensk röd och vit boskap (SRB) och Svensk låglandsboskap (SLB) med speciell tonvikt på dödfödslar.*

Under 3 på varann följande säsonger studerades kalvningsförlopp, placenta-variabler och hormonella profiler från totalt 98 sena dräktigheter av SRB respektive SLB djur. Både kvigor (paritet 1) och kor (paritet 2) studerades. Nittiofyra enkelbörd och 4 tvillingbörd blev resultatet; och i enkelbördsdjuren registrerades 8 dödfödslar, 7 svaga kalvar, 3 för tidiga förlossningar och en abort. Dödfödselfrekvensen för kvigor av SRB och SLB ras var 5% (2/42) respektive 19% (5/26). Endast en dödfödd kalv (1/15) sågs i kogrupper av SRB ras och ingen (0/11) av SLB korna. Hos både kvigor och kor där kalvarna levde och kalvningsförloppet var normalt var placentalomfjockleken, uppmätt med ultraljudsteknik, konstant. Antalet fetala kotyledoner varierade individuellt mellan djur, men totalantalet var lägre i fosterhinnorna från SRB djur jämfört med SLB djur,  $69 \pm 19$  mot  $88 \pm 29$

( $p < 0.05$ ). Inga morfologiska eller numeriska skillnader kunde observeras i placentomtjockleken mellan djur som förlöste dödfödda eller svaga kalvar jämfört med normala levande kalvar. De djur som hade en okomplicerad förlossning och inga problem med kalvens vitalitet hade, oavsett ras och paritet, helt jämförbara nivåer av progesteron (P4),  $\text{PGF}_{2\alpha}$  metabolit (PG-metabolit), kortisol, östronsulfat (EISO4) och sk. 'pregnancy associated glycoproteins' (PAGs). Hos de 3 SRB djuren som fick dödfödda kalvar var

EISO4 nivåerna förhöjda i jämförelse med de okomplicerade dräktigheterna, men lägre nivåer uppmättes hos 3 SLB kvingor med dödfödda kalvar. Nivåerna av PAGs var låga och konstanta hos en SRB ko (dödfödd kalv), en SRB kvinga (för tidig förlossning), 4 djur med tvillingbörd samt hos en SRB ko som aborterade. Våra resultat visar en mycket hög dödfödselfrekvens speciellt hos SLB kvingorna och att djur med förlossningskomplikationer visade avvikande hormonprofiler för EISO4 och PAGs.

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